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(54) Title: USE OF MHC-II BINDING AND/OR MHC-II MIMICKING MOLECULES FOR THE PREVENTION AND/OR TREATMENT OF INFLAMMATORY DISEASES

(57) Abstract

The invention relates to MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between an activation stimulus for MHC-II bearing cells, such as phagocytes and cell-bound MHC-II molecules, in the interaction between lipopolysaccharide (LPS) or LPS in a complex with other molecules, such as CD14 and LBP, and cell-bound MHC-II molecules, or in the interaction between products from Gram-positive bacteria or complexes of products from Gram-positive bacteria with molecules such as CD14, and cell-bound MHC-II molecules. The MHC-II binding molecule may be any anti-MHC-II antibody or fragment thereof, or any molecule derived from such an antibody such as humanized, bispecific or other engineered molecules and the like. The MHC-II binding molecule may be selected from the group consisting of CD14, fragments thereof, modified versions thereof, or peptides having MHC-II binding properties.

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USE OF MHC-II BINDING AND/OR MHC-II MIMICKING MOLECULES FOR THE PREVENTION AND/OR TREATMENT OF INFLAMMATORY DISEASES

The present invention relates to the use of specific molecules for interfering in the interaction between toxins like lipopolysaccharide (LPS) alone or as a complex with other molecules, such as CD14 and LBP, and its transducer molecule. The invention further relates to the use of these molecules in the prevention and treatment of inflammatory diseases, like septic shock.

Lipopolysaccharide (LPS) is a constituent of the cell wall of Gram-negative bacteria. Infection with Gram-negative bacteria can result in a life-threatening disease, which is caused by specific binding of LPS to phagocytes, like monocytes, macrophages and granulocytes, which are thereby activated and secrete various cytokines, including tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6, IL-8, and other mediators of inflammation. These substances, either by direct action or by activation of secondary mediators, initiate a cascade of events resulting in disorders of the coagulation system, vasodilatation, multi-organ failure and, ultimately, septic shock (4, 5).

In general the marked activation of phagocytic cells, resulting in secretion of a multitude of inflammatory mediators, is a central event in the pathogenesis of a pathologic condition called Systemic Inflammatory Reaction Syndrome (SIRS). Besides activation by LPS, SIRS can develop as a result of various other clinical conditions, such as infection with bacteria or viruses, trauma, burns, pancreatitis, graft-versus-host and host-versus-graft disease, hemophagocytosis and many more. Toxic shock, caused by exotoxins, like staphylococcal toxin A, from Gram-positive bacteria, is one example of an inflammatory disease. Other exotoxins, also known as superantigens, are staphylococcal toxin B and streptococcal toxins.

It has been demonstrated that LPS binds to the glycosylphosphatidylinositol (GPI)-anchored monocytic anti-

gen CD14. CD14 is present on the surface of monocytes, macrophages and granulocytes, but is also found in a soluble form without the GPI anchor in the serum of healthy individuals. Furthermore it has been demonstrated that activation of monocytes by LPS can be inhibited by anti-CD14 monoclonal antibodies. It was therefore suggested that CD14 would serve as a receptor for LPS (1) and mediates the effects of LPS to the cytoplasm. However, CD14-negative cells can also respond to LPS (2, 7, 8).

Furthermore, it became known that CD14 is a glycosylphosphatidylinositol (GPI)-anchored molecule, lacking a transmembrane and cytoplasmic domain (9). Thus CD14 can not transduce a signal to the cytoplasm. It is a widely accepted hypothesis that GPI-linked proteins require associated transmembrane molecules for signal transduction. Thus, the real transducer molecule for LPS and possibly other SIRS stimuli, has not yet been identified.

In the case of cell activation by LPS, molecules other than CD14 have to be invoked to explain cellular activation by LPS (10). It was proposed that LPS forms a complex with either membrane-bound or soluble CD14 and the LPS binding protein (LBP). Other serum-derived molecules may also participate in this complex. The complex interacts with an as yet unidentified molecule on the cell surface, leading to the activation of the cells.

CD14 has been described as playing a key role in initiating cell activation by bacterial envelope products from Gram-positive as well as Gram-negative organisms (13). Again, other membrane-bound or serum-derived molecules may be involved in the interaction with the cell-surface molecule which leads to cellular activation.

According to the present invention it has now been found that Major Histocompatibility Complex II (MHC-II) molecules are required for activation of cells by LPS, either by serving as receptor and/or as a signal transducing element for molecular complexes of LPS and molecules like CD14 and LBP. In humans MHC is known as Human Leucocyte Antigen (HLA). It has been shown that LPS-responsiveness

depends on expression of MHC class II-molecules on the cell surface. A cell line, referred to herein as "THP-1^{MHC+}", is an MHC class II expressing monocytic cell line, described as THP-1 by Tsuchiya et al. (3). A second cell line, referred to herein as "THP-1.6^{MHC-}", is an MHC class II-negative monocytic cell line derived from THP-1^{MHC+} by spontaneous mutation. THP-1^{MHC+} cells secrete cytokines in response to LPS, whereas THP-1.6^{MHC-} cells do not. CD14-positive, MHC II-negative human peripheral blood mononuclear cells (PBMC) are irresponsive to LPS, too. MHC class II-expression and LPS-responsiveness can be restored by transfecting THP-1.6^{MHC-} cells with CIITA, a cDNA encoding a nuclear factor essential for the expression of MHC-II molecules on the cell surface.

The transduction of other SIRS stimuli to the cell may also be mediated by MHC-II molecules. It has already been demonstrated previously that exotoxins also bind to MHC-II molecules on the cell surface. The activity of other Gram positive products is mediated at least in part by CD14, and it is thus likely that the complex of these products with CD14 also interacts with MHC-II molecules to activate cells.

The prevention and/or treatment of systemic inflammatory reaction syndrome may thus be performed by interfering in the interaction between the complex of LPS and other molecules, like CD14 and LBP (indicated hereinbelow as "CD14/LPS/LBP complex"), and cell-bound MHC-II. According to the invention this interference may be effected in two different ways.

First the binding of the CD14/LPS/LBP complex to cell-bound MHC-II may be blocked by MHC-II binding molecules, such as anti-MHC-II antibodies, CD14 or peptides derived thereof. This type of molecule competes with the CD14/-LPS/LBP complex and thus prevents the complex from binding but does not itself activate the MHC-II. The transducer function of MHC-II is then blocked.

Second the circulating LPS or CD14/LPS/LBP complex may be captured by MHC-II mimicking molecules. Complexes binding to soluble MHC-II or MHC-II-like molecules are no

longer able to bind to the cell-bound MHC-II. Thus activation of the cell is prevented.

MHC-II binding molecules comprise any molecule that is capable of blocking binding of LPS or the CD14/LPS-complex to MHC-II. In practice this will comprise anti-MHC-II antibodies, both monoclonal and polyclonal antibodies, directed to the CD14/LPS or LPS binding site of a cell. Antibody fragments are also suitable as MHC-II binding molecules.

MHC-II mimicking molecules are meant to comprise both soluble MHC-II molecules themselves as well as any other molecule that is capable of blocking the MHC-II binding site on LPS or the CD14/LPS complex. Molecules of this type may comprise complete MHC-II molecules or fragments or subunits thereof. Furthermore peptides capable of binding to LPS or the LPS/CD14/LBP complex without activating the MHC-II may be useful. Such peptides may be at least homologous to MHC-II and comprise suitable D-amino acids providing the peptide with antagonistic properties. The molecules may be in a soluble form or coupled to the surface of a carrier.

Based on the information given in this application, the skilled person will be able to identify suitable binding and/or mimicking molecules.

This type of molecule may originate from any suitable source, either human or other, and be prepared by various means, such as isolation from the supernatant of a cell culture of MHC-II positive cells or from a cell lysate. An especially preferred isolation method is immunoaffinity chromatography. Suitable molecules may also be prepared by gene technology, by protein chemical methods or any other suitable method.

According to the invention these two types of molecules may be used in the prevention and/or treatment of inflammatory diseases, like septic shock, graft-versus-host disease after organ transplantations, like bone marrow transplantations, graft rejection reactions, inflammatory reactions resulting from burns, accidents, infections of the pancreas etc..

The invention is also suitable for the prevention and/or therapy of other inflammatory reactions occurring e.g. after surgery, like capillary leak syndrome, allergic diseases, autoimmune diseases, like Lupus Erythematodes (LE) and sub-forms thereof, sclerodermia and its sub-forms, eosinophilic fasciitis, Sjögren Syndrome, polymyositis, dermatomyositis, periarteritis nodosa, Wegener's granulomatosis, arteritis temporalis, polymyalgia rheumatica etc., rheumatoid disorders, like rheumatoid arthritis, juvenile chronic arthritis, Felty syndrome, Caplan syndrome, ankylosating spondylitis (Marie-Strümpell-Bechterew disease), psoriasis, Reiter syndrome, Behçet syndrome.

Other diseases that may be treated according to the invention and at least partially result from autoimmune mechanisms are inter alia diabetes mellitus, morbus Crohn, colitis ulcerosa, digestive tract ulcers, renal infections, like glomerulonephritis and nephritis, arteriosclerotic disorders, multiple sclerosis, Alzheimer's disease, hyperthyreosis, hypothyreosis.

The invention may also be used in inflammatory reactions in one or more human organs associated with oncological disorders, such as leukemia, blood cell tumors, carcinoma, fibroma, sarcoma, and various types of histiocytosis.

The invention may also be used for the prevention and/or treatment of viral diseases such as AIDS. LPS-stimulation is known to increase intracellular Human Immunodeficiency Virus (HIV) replication. Blocking the stimulation by LPS by MHC-II binding and/or mimicking molecules of the invention will thus remove this replication stimulus. MHC-II binding and/or mimicking molecules of the invention may therefore be used to impart a protective effect on HIV-infected cells by preventing the stimulation of viral replication by cell-activating stimuli.

Based on the data presented in the examples the following model for activation of cells by LPS could be imagined. On the one hand, LPS may bind to membrane-bound CD14 (mCD14), an interaction accelerated by Lipopolysaccha-

ride Binding Protein LBP (14). The complex of LPS and GPI-anchored mCD14 and possibly LBP would then interact with the transmembrane MHC class II-molecules, resulting in signal transduction. This situation is shown in figure 3A. On the other hand, LPS together with LBP may bind to soluble CD14 (sCD14) present in the serum, and this complex may then bind to MHC class II-molecules on the surface of CD14-negative cells, resulting in activation of CD14-negative, but MHC class II-positive cells. This situation is illustrated in the figure 3B. Although contact is shown between CD14 and MHC-II, LBP and LPS may also participate in this interaction as may other as yet unidentified molecules. The activation stimulus may also be components of Gram-positive bacteria, although in this case the role of LBP has not been determined. The possibility that some activation stimuli act directly on the MHC-II molecules without forming a complex with CD14 or LBP is herewith not excluded. It is now established that stimulation is mediated by MHC-II molecules. Blocking or mimicking these molecules thus will prevent the transduction of the activation stimulus.

The invention further relates to the MHC-II binding molecules, to the MHC-II mimicking molecules and to pharmaceutical compositions comprising either or both types of molecules. Pharmaceutical compositions, comprising one or more MHC-II binding molecules and/or MHC-II mimicking molecules as the active ingredient for interfering in the interaction between an activation stimulus, such as LPS, for cells expressing MHC-II molecules, such as phagocytes, and cell-bound MHC-II molecules have the form of powders, suspensions, solutions, sprays, emulsions, infusions, inhalation compositions, unguents or creams and can be used for local application, intranasal, rectal, vaginal and also for oral, parenteral (intravenous, intradermal, intramuscular, intrathecal etc.) or transdermal administration, administration by means of inhalation etc.. Pharmaceutical compositions of the invention can be prepared by combining (i.e. by mixing, dissolving etc.) the active compound(s) with pharmaceutically acceptable excipients with neutral character

(such as aqueous or non-aqueous solvents, stabilizers, emulsifiers, detergents, additives), and further if necessary coloring agents and flavoring agents. The concentration of the active ingredient in a pharmaceutical composition can vary between 0.001% and 100%, depending on the nature of the treatment and the method of administration. The dose of the active ingredient that is administered also depends on the specific application and route of administration, but may for example vary between 0.01 µg and 1 mg per kg body-weight, preferably between 0.1µg and 100 µg per kg body-weight.

The invention will be illustrated with reference to the following examples, which are not intended to limit the scope of the invention.

EXAMPLES

EXAMPLE 1

Introduction

The hypothesis that MHC-II is the transducer and/or receptor for a complex including LPS, CD14, LBP and possibly other molecules in the activation of phagocytic cells by LPS was tested by comparing the secretion of cytokines by MHC class II-positive (HLA-DR) and MHC class II-negative cell lines upon stimulation with LPS. The secretion of cytokines is indicative of activation of the cells.

Materials and methods

THP-1^{MHC+} is a CD14-negative, MHC class II-positive monocytic cell line of human origin (3). THP-1.6^{MHC-} is a spontaneously derived, MHC class II-negative mutant of THP-1^{MHC+}, cloned by repeated limiting dilutions.

HLA-DR expression was reconstituted by transfection of THP-1.6^{MHC-} cells with CIITA (class II transactivator; a nuclear protein essential for the expression of MHC class II-proteins (12)) to yield THP-1.6^{MHC-}CIITA.

Expression of HLA-DR and CD18 on the surface of THP-1^{MHC+} cells (wild-type), THP-1.6^{MHC-} cells (mutant cell

line) and THP-1.6^{MHC-}CIITA (THP-1.6^{MHC-} cells transfected with CIITA) was assessed by flow cytometry.

The various cell lines were cultured at 10^6 cells/ml in medium supplemented by 10% FCS and stimulated with LPS in doses of 0, 1, 10, 100 ng/ml and 1 µg/ml. After 24 hours, the supernatants were harvested and assessed by ELISA for their content of TNF-α and IL-8.

Results and discussion

Viability of the cells was not compromised by the LPS treatment. More than 95% of the cells were viable as shown by trypan blue staining.

While expression of CD18 was similar in the three cell lines, THP-1.6^{MHC-} cells expressed significantly lower levels of HLA-DR than THP-1^{MHC+} cells.

Upon stimulation of THP-1^{MHC+} cells with LPS the cells responded by secretion of TNF-α and IL-8 (fig. 1). By contrast, the HLA-DR-negative cell line THP-1.6^{MHC-} could not be induced to secrete TNF-α nor IL-8 by stimulation with LPS, even using high doses of LPS. However, HLA-DR-expression and LPS-responsiveness were both restored by transfection with CIITA. It was concluded that expression of HLA-DR molecules was required for activation of monocytic cells by LPS.

EXAMPLE 2

Introduction

To confirm the finding of example 1 the results obtained with the cell line system were verified using cells from a patient with MHC class II-deficiency, a rare inherited disease manifesting as severe combined immuno-deficiency in early childhood. CD14-expression is not affected in these patients. PBMC of a patient with MHC class II-deficiency and of a control individual were cultured in the presence of increasing doses of LPS and the levels of TNF-α and of IL-1 were measured in the supernatants of these cultures.

Materials and methods

Peripheral Blood Mononuclear Cells (PBMC) were taken from a patient with MHC-II deficiency and from a healthy control.

The expression of HLA-DR on the surface of both PBMC of the patient (hatched histogram, left panel of Fig. 2) and of the healthy control (grey histogram) was assessed by direct immunofluorescence staining and flow cytometry.

PBMC from the patient (thick line) and from the control (thin line) were cultured at 10^6 cells/ml in medium supplemented by 10% normal human AB-positive serum and stimulated with 0, 1, 10 and 100 ng/ml of LPS. After 24 hours the supernatants were harvested and assessed by ELISA for their content of TNF-α.

Results and discussion

PBMC from healthy individuals secreted TNF-α and IL-1 in response to LPS, as expected. However, the patient's MHC class II-deficient PBMC did not secrete significant levels of cytokines in response to LPS (fig. 2). Thus it was shown that the requirement for expression of MHC class II-molecules was not limited to the THP-1^{MHC+} family of cell lines.

The data show that membrane-bound CD14 is neither required for activation of cells by LPS (THP-1^{MHC+} cells do not express CD14) nor sufficient (PBMC from MHC class II-deficient patients express normal levels of CD14).

However, the experiments outlined above do not address the role of soluble CD14. In all these experiments media supplemented with serum containing soluble CD14 have been used. The experiments were therefore repeated, culturing THP-1^{MHC+}, THP-1.6^{MHC-} and THP-1.6^{MHC-}CIITA cells in serum-free medium. Treatment with increasing doses of LPS did not result in secretion of significant levels of TNF-α.

The results indicate that MHC class II-molecules are crucially involved in LPS-responsiveness. HLA-DR negative cells could not secrete cytokines upon stimulation

with LPS. One could argue that lack of response to LPS is related to a factor closely associated with MHC class II and regulated by CIITA, too, rather than lack of MHC class II-expression per se causing depressed LPS-responsiveness. However, the defect in the response to LPS is not limited to the secretion of one cytokine alone since secretion of TNF-α as well as IL-1 and IL-8 was depressed. Furthermore no molecule regulated by CIITA other than MHC class II has been found to date, despite extensive investigations. Finally, the patient's disease is not due to a defect related to CIITA. Transfection of Epstein Barr Virus (EBV)-transformed B-cells from this patient did not restore expression of MHC class-II molecules.

EXAMPLE 3

Introduction

The physical interaction between MHC class II-molecules and CD14 was demonstrated in three different ways.

First, lysates of MHC II positive and MHC II negative cell lines were incubated with MHC II specific or control antibodies and radioactive CD14 ($CD14^*$) or a radioactive control molecule. Complexes between MHC II and $CD14^*$ can only be precipitated by interaction of protein A-agarose and MHC II specific antibodies. No radioactivity should be found in the precipitate when the control molecule or the control antibody is used. If no MHC II is present, no radioactivity may be found in the precipitate.

Second, MHC II was incubated with radioactive CD14. Presumably MHC II/$CD14^*$ complexes will be formed. In theory, these complexes may be precipitated by means of antibodies that are specific for either of the two partners in the complex, and protein A-agarose.

Third, it was tested whether anti-CD14 antibodies could block the interaction of MHC II and $CD14^*$.

The principle of these experiments is further illustrated in figure 4.

Methods

1. Production of radioactive CD14

Recombinant CD14 has been produced by in vitro transcription/translation from a full length CD14 cDNA, that was prepared by means of PCR techniques and the nucleotide sequence of which was verified, using the TNT T7 coupled reticulocyte lysate system following the manufacturer's protocol (Promega, Switzerland) in the presence of ^{35}S-methionine. As control, radioactively labeled luciferase has been produced by in vitro transcription/translation using the same system.

2. Production of cell lysates

So-called 293 cells are derived from human embryonal kidney transformed with human adenovirus type 5 DNA (ATCC designation CRL 1573); wild-type 293 cells do not express MHC class II-molecules as detected by FACS-analysis. These were taken as MHC II-negative cells. 293 cells transfected with cDNA from the α-, β and invariant (i) chains of human MHC class II were obtained form Dr. Jacques Neefjes, Netherlands Cancer Institute, Amsterdam. These do express MHC II and are therefore used as MHC II positive cells. Lysates from MHC class II-positive and MHC class II-negative cell lines were produced as follows.

5×10^6 cells were lysed in a Petri dish in 1 ml of lysis buffer (Boehringer Mannheim, cellular labeling and immunoprecipitation kit). After 30 minutes lysed cells were collected and subjected to sonification (3x15 seconds), followed by 30 minutes of incubation on ice and subsequent centrifugation. The supernatants were subjected to immunoprecipitation as described below.

3. Immunoprecipitation in Experiment 1

Immunoprecipitation was performed using the reagents and following the protocol from the "Cellular labeling and immunoprecipitation kit" form Boehringer Mannheim (Switzerland). In brief, the samples were precleared using 50µl protein A-agarose suspension. After removal of this protein

A-agarose by centrifugation the supernatants were incubated with the following antibodies: L243 (anti-HLA-DR, ATCC designation HB 55); 1B5 (anti-MHC class II, obtained from Dr. Jacques Neefjes, Netherlands Cancer Institute, Amsterdam); and anti-CD3 (Pharmingen, San Diego, USA/AMS Biotechnology, Switzerland). After 1 hour, 50 µl protein A-agarose was added, and the samples were incubated for 3 hours followed by 6 washes.

Then the pellets were resuspended in 30 µl of buffer (50 mM Tris, pH 7.5, 20 mM NaCl, 1 mM EDTA, 2 mM PMSF), and 5 µl from the solution obtained in the in vitro transcription/translation reaction containing radioactively labeled CD14 were added, and this mixture was incubated for 30 minutes at room temperature. After centrifugation, the pellets were washed 2x with wash buffer #2 followed by 2 washes with wash buffer #3 (buffers #2 and #3 originate from the cellular labeling and immunoprecipitation kit of Boehringer Mannheim, Switzerland). The pellets were then dissolved and boiled in standard SDS-PAGE loading buffer and subjected to gel electrophoresis on a 10% SDS polyacrylamide-gel, followed by autoradiography.

4. Purification of MHC class II-molecules

MHC class II-molecules were extracted and purified from THP-1 cells as described by Gorga et al. (15) with minor modifications. L243 (anti-HLA-DR, ATCC designation HB 55) was immobilized on a protein A-Sepharose 4B column by crosslinking with dimethylpimelimidate. THP-1 cells were lysed on ice in Tris-HCl pH 8.0 containing 0.1 mM PMSF. All subsequent steps were carried out at 4°C. The lysate was centrifuged at 3000xg and the pellet washed until the supernatant was clear. The pooled supernatants were centrifuged at 160000xg for 40 min and the pellet redissolved by the addition of Nonidet P40 to 4% final concentration. After centrifugation at 160000xg for 2 hours, the supernatant was applied to series of columns in the following order: Sepharose 4B (10ml), normal rabbit serum-Affigel 10 (10ml), Protein A-Sepharose 4B (2ml) L243-

Sepharose 4B (12ml). The columns were washed with 10 mM Tris-HCl/0.1% Nonidet P40 pH 8.0 (5 vols.); 10 mM MOPS/140 mM NaCl/0.1% deoxycholate pH 8.0 (2 vols.); 10mM Tris-HCl/0.1% deoxycholate pH 8.0 (4 vols.). The L243 column was then disconnected from the other columns and eluted rapidly with 50 mM glycine/0.1% deoxycholate pH 11.5. 10 ml fractions were collected and adjusted to pH 7.0 to 8.0 as soon as they were eluted with 2M glycine pH 2.0. The eluted HLA-DR molecules were concentrated by ultrafiltration with 30 kDalton cut-off membranes. After washing three times in Tris-HCl/0.1% deoxycholate pH 8.0, the protein was rediluted in the same buffer.

5. Immunoprecipitation in Experiment 2

5 µl from the solution containing radioactively labeled CD14 obtained in the in vitro transcription/ translation reaction were mixed with 1 µl of the solution containing approximately 10ng MHC class II-molecules and incubated for 30 minutes at room temperature. Then, the antibodies were added and the samples were incubated for 1 hour at 4°C. After addition of 50 µl protein A-agarose the samples were incubated for another 3 hours at 4°C. After centrifugation, the pellets were washed 2x with wash buffer #2 followed by 2 washes with wash buffer #3 (cellular labelling and immunoprecipitation kit, Boehringer Mannheim, Switzerland). The pellets were then dissolved and boiled in standard SDS-PAGE loading buffer and subjected to gel electrophoresis on a 10% SDS polyacrylamide-gel, followed by autoradiography.

6. Immunoprecipitation in Experiment 3

Before adding the radioactively labeled CD14 to the immunoprecipitation samples as described for the other immunoprecipitation experiments, the solution containing CD14 was incubated with a cocktail of anti-CD14 antibodies for 10 minutes at room temperature. The cocktail consisted of 10 µg of each of the following anti-CD14 antibodies: RM052 (Immunotech, Switzerland); LeuM3 (Beckton & Dickinson,

Switzerland); MY4 (Coulter, Swtizerland); Tük 4 (Dako, Switzerland); and 100 µl of the supernatant of the following anti-CD14 hybridomas: 3C10, 63D3 (both obtained from ATCC). As a control, PBS in the same volume as the volume of the antibody cocktail, was added to 5µl of the solution containing radioactively labeled CD14.

The antibodies used for the immunoprecipitation were: MY4 (anti-CD14) and L243 (anti-MHC II).

Results

EXPERIMENT 1

Demonstration by coprecipitation of MHC class II-molecules and CD14 using lysates from MHC class II-positive and MHC class II-negative cells

The results of this experiment are shown in figure 5. On the left side of the panel, the results from the experiments performed using lysate from 293-cells transfected with MHC class II (i.e. cotransfected with the α, ß, and i-chain of MHC class II) are depicted, on the right side the results using lysate of the MHC class II-negative 293 wild-type cells are visible.

In lanes 1 and 2 only faint bands presumably corresponding to radioactive CD14 (lane 1) respectively to luciferase (lane 2) were unspecifically precipitated by the control antibody anti-CD3. Bands corresponding to radioactively labeled CD14 ($CD14^*$) are clearly visible in lanes 3 and 5; two different antibodies recognizing MHC class II-molecules (lane 3: L243; lane 5: 1B5) have (co-)precipitated $CD14^*$. The coprecipitating effect of these anti-MHC class II antibodies is specific for CD14, since no significant amount of a radioactively labeled control protein (luciferase) is coprecipitated by these antibodies (lanes 4 and 6). The coprecipitation of CD14 by anti-MHC class II antibodies is dependent on the presence of MHC class II-molecules, since there is no precipitation of CD14 by anti-MHC class II antibodies in the absence of MHC class II-molecules (using lysate from MHC class II-negative 293 cells, lanes 7-9).

EXPERIMENT 2

Demonstration by coprecipitation of MHC class II-molecules and CD14 using MHC class II-molecules purified by an immuno-affinity column

The results are shown in figure 6. On the left side of the panel the results from the immunoprecipitation experiments performed in the presence of purified MHC class II-molecules are depicted, on the right side the results from the experiments performed in the absence of purified MHC class II-molecules, i.e. using the buffer as negative control, are visible.

In lanes 1 and 4 faint bands corresponding to CD14 unspecifically precipitated by the control antibody (anti-CD3) are visible. In lane 3 a band corresponding to CD14 appears upon precipitation by an anti-CD14 antibody. In lane 2 the band corresponding to CD14 is of greater intensity than in lane 3, although in this experiment precipitation has been performed with an anti-MHC class II-molecules. In the absence of purified MHC class II-molecules, CD14 is strongly precipitated by anti-CD14 antibodies (lane 6), whereas coprecipitation of CD14 with an anti-MHC class antibody does not exceed background level (lane 5).

EXPERIMENT 3

The physical interaction between CD14 and MHC class II-molecules can be inhibited by anti-CD14 antibodies

The results are shown in figure 7. Lanes 1 and 5 show that the anti-CD14 antibody MY4 precipitates the radioactively labeled CD14 produced by *in vitro* transcription/translation, independently of the presence (lane 1) or absence (lane 5) of MHC class II-molecules (control). CD14 is strongly precipitated by L243, an anti-MHC class II antibody, provided MHC class II molecules are present (lane 2), but only in background amounts in the absence of MHC class II-molecules (lane 6). If the CD14 is treated first with a cocktail of anti-CD14 antibodies, previously to being mixed with the cell lysate containing MHC II-molecules, CD14 cannot be precipitated by anti-MHC class II antibodies. Lane

4 shows that a buffer (control) has no effect on the coprecipitation of CD14 by anti-MHC class II antibodies. Lanes 7 and 8 show the results of the same experiments as in lanes 3 and 4 but performed in the absence of MHC class II-molecules.

EXAMPLE 4

Introduction

To demonstrate the role of MHC II in the activation of cells in vivo MHC class II knock-out mice were used. If MHC II is involved in the activation mechanism by LPS, mice lacking MHC II should not show the usual physiological effects of LPS stimulation. A similar experiment was performed in vitro by using blood of the same mice.

Methods

For the in vivo experiment 100 µg of LPS (E.coli 0111:B4, diluted in sterile 0.9% NaCl) were injected intravenously in wild-type C57BL/6 and B6-Aa^0/Aa^0 MHC class II knock-out mice (Hoffmann-La Roche; ref. 16). After 2 hours the mice were sacrificed and bled sterily. The blood was allowed to coagulate at room temperature and was centrifuged for 5 minutes at 13.000 rpm. Then the serum was removed. The content of TNF-α, a marker for activation of phagocytes, was determined in the serum by ELISA (Biosource). Figure 8 shows the results.

For the in vitro experiment heparinized whole blood from wild-type MHC II positive C57BL/6 mice (black circles in Figure 9) and B6-Aa^0/Aa^0 mice (open squares), in which MHC class II-expression is lacking after targeted disruption of the MHC class II gene Aa^1, was incubated in the presence of 0, 0.1, 1, 10 and 100 ng/ml LPS. After 4 hours incubation, the level of TNF-α was assessed in plasma by ELISA (Biosource). The results are shown in figure 9.

Clearly the TNF-α secretion is higher in mice or cells expressing MHC class II molecules.

EXAMPLE 5

Introduction

The in vivo effect of MHC II-binding and MHC II-mimicking molecules was assessed as follows.

Method

Wild-type C57B1/6 mice were weighed individually and injected with an LD_{50} determined in preliminary experiments. Before injection of LPS mice were pretreated with:

Group 1: soluble MHC class II molecules in a dose range from 1 μg to 1 mg/kg body weight;

Group 2: anti-MHC class II antibodies in the same dose range; and

Group 3: saline only (control group);

As a control three other groups got soluble MHC class II, anti-MHC II or saline, respectively without LPS challenge afterwards.

Survival was monitored for 7 days. Mice were observed on a regular basis during the first 48 hours to note symptoms. One sub-group of mice was sacrificed and bled before LPS-induced death occurred and levels of TNF-α were determined in the serum from these animals by ELISA.

Results

Mortality as well as TNF-α serum levels were significantly decreased in the groups of mice treated with soluble MHC class II molecules or anti-MHC II antibodies (results not shown) as compared. The control group treated with MHC class II molecules or anti-MHC II antibodies but without LPS challenge did not show significant differences from the mice treated with saline without LPS challenge.

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CLAIMS

1. MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound MHC-II molecules.

2. MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between an activation stimulus for phagocytes and cell-bound MHC-II molecules.

3. MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between lipopolysaccharide (LPS) or LPS in a complex with other molecules, such as CD14 and LBP, and cell-bound MHC-II molecules.

4. MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between products from Gram-positive bacteria or complexes of products from Gram-positive bacteria with molecules such as CD14, and cell-bound MHC-II molecules.

5. MHC-II binding and/or MHC-II mimicking molecules as claimed in any one of the claims 1-4, wherein the MHC-II binding molecule is an anti-MHC-II antibody or fragment thereof, or any molecule derived from such an antibody such as humanized, bispecific or other engineered molecules and the like.

6. MHC-II binding and/or MHC-II mimicking molecules as claimed in any one of the claims 1-4, wherein the MHC-II binding molecule is selected from the group consisting of CD14, fragments thereof, modified versions thereof, or peptides having MHC-II binding properties.

7. MHC-II binding and/or MHC-II mimicking molecules as claimed in claim 1, 2, 3 or 4, wherein the MHC-II mimicking molecule is selected from the group consisting of soluble complete MHC-II, one or more soluble subunits of MHC-II, fragments of complete MHC-II or subunits thereof, modified versions of complete MHC-II or subunits thereof,

peptides having the LPS or LPS/CD14/LBP complex binding properties of MHC-II or subunits thereof.

8. MHC-II binding and/or MHC-II mimicking molecules as claimed in claim 1, 2, 3, 4 or 7 wherein the MHC-II mimicking molecule is coupled to a carrier.

9. MHC-II binding and/or MHC-II mimicking molecules as claimed in any one of the claims 1-8 for use in the prevention and/or treatment of inflammatory diseases; septic shock; graft-versus-host disease after organ transplantations, like bone marrow transplantations; graft rejection reactions; inflammatory reactions in one or more human organs resulting from burns, accidents, infections of the pancreas, such as adult respiratory distress syndrome (ARDS) etc.; inflammatory reactions occurring in one or more human organs after surgery, like capillary leak syndrome; allergic diseases in one or more human organs; inflammatory reactions in one or more human organs associated with autoimmune diseases, like Lupus Erythematodes (LE) and sub-forms thereof, sclerodermia and its sub-forms, eosinophilic fasciitis, Sjögren Syndrome, polymyositis, dermatomyositis, periarteritis nodosa, Wegener's granulomatosis, arteritis temporalis, polymyalgia rheumatica etc.; inflammatory reactions in one or more human organs associated with rheumatoid disorders, like rheumatoid arthritis, juvenile chronic arthritis, Felty syndrome, Caplan syndrome, ankylosating spondylitis (Marie-Strümpell-Bechterew disease), psoriasis, Reiter syndrome, Behçet syndrome; inflammatory reactions in one or more human organs associated with diseases which at least partially result from autoimmune mechanisms, such as diabetes mellitus, morbus Crohn, colitis ulcerosa, digestive tract ulcers, renal inflammations, like glomerulonephritis and nephritis, arteriosclerotic disorders, multiple sclerosis, Alzheimer's disease, hyperthyreosis, hypothyreosis; inflammatory reactions in one or more human organs associated with oncological disorders, such as leukemia, blood cell tumors, carcinoma, fibroma, sarcoma, and various types of histiocytosis; and viral diseases such as AIDS.

10. MHC-II binding molecules.

11. MHC-II mimicking molecules.

12. Pharmaceutical compositions comprising as the active ingredient MHC-II binding and/or MHC-II mimicking molecules together with a suitable excipient.

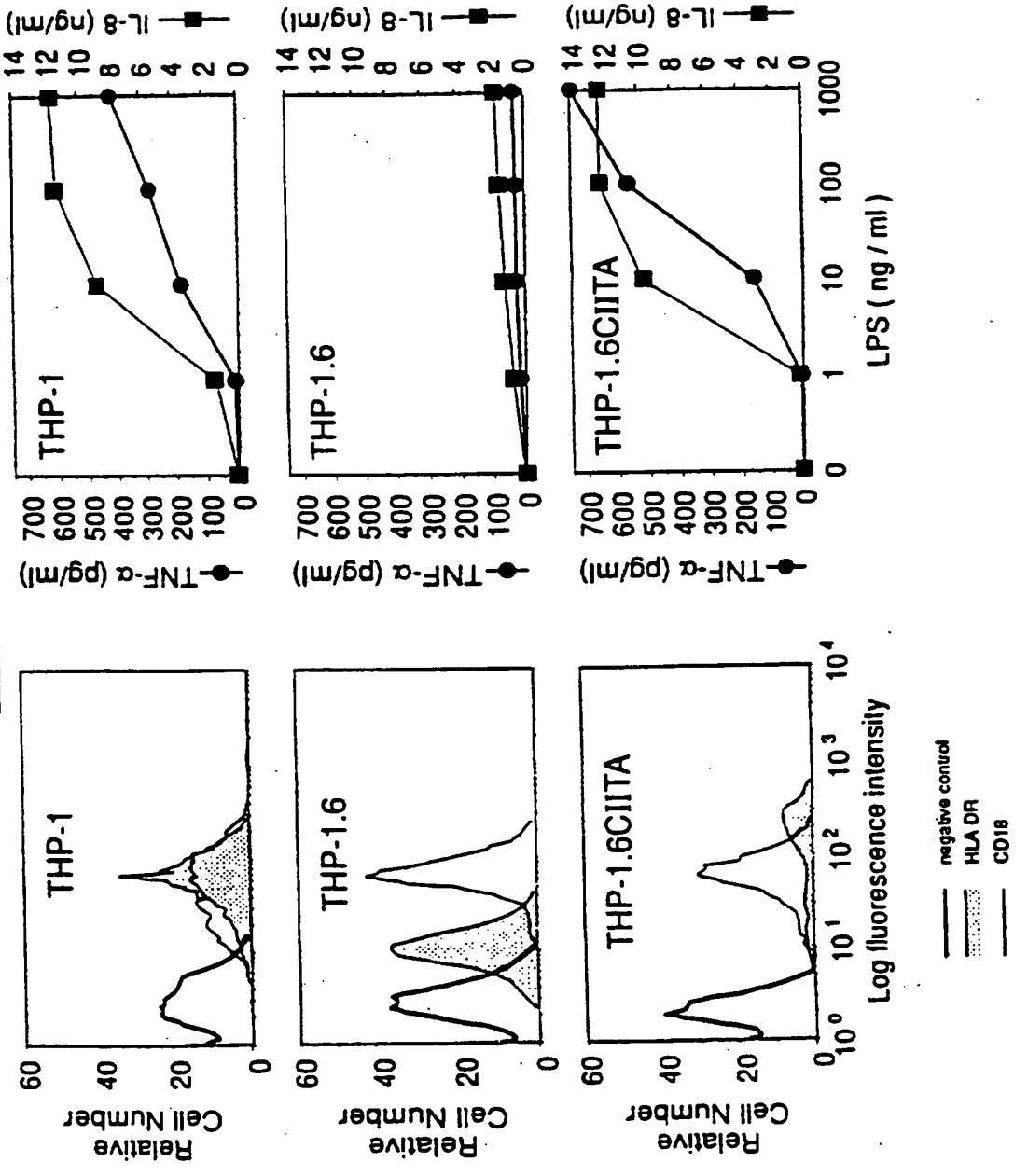
13. Use of MHC-II binding and/or MHC-II mimicking molecules for the preparation of a pharmaceutical composition for interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound MHC-II molecules.

14. Use of MHC-II binding and/or MHC-II mimicking molecules for the preparation of a pharmaceutical composition for interfering in the interaction between an activation stimulus for phagocytes and cell-bound MHC-II molecules.

15. Use of MHC-II binding and/or MHC-II mimicking molecules for the preparation of a pharmaceutical composition for interfering in the interaction between lipopolysaccharide (LPS) or LPS in a complex with other molecules, such as CD14 and LBP, and cell-bound MHC-II molecules.

16. Use of MHC-II binding and/or MHC-II mimicking molecules for the preparation of a pharmaceutical composition for interfering in the interaction between products from Gram-positive bacteria or complexes of products from Gram-positive bacteria with molecules such as CD14, and cell-bound MHC-II molecules.

FIG.1



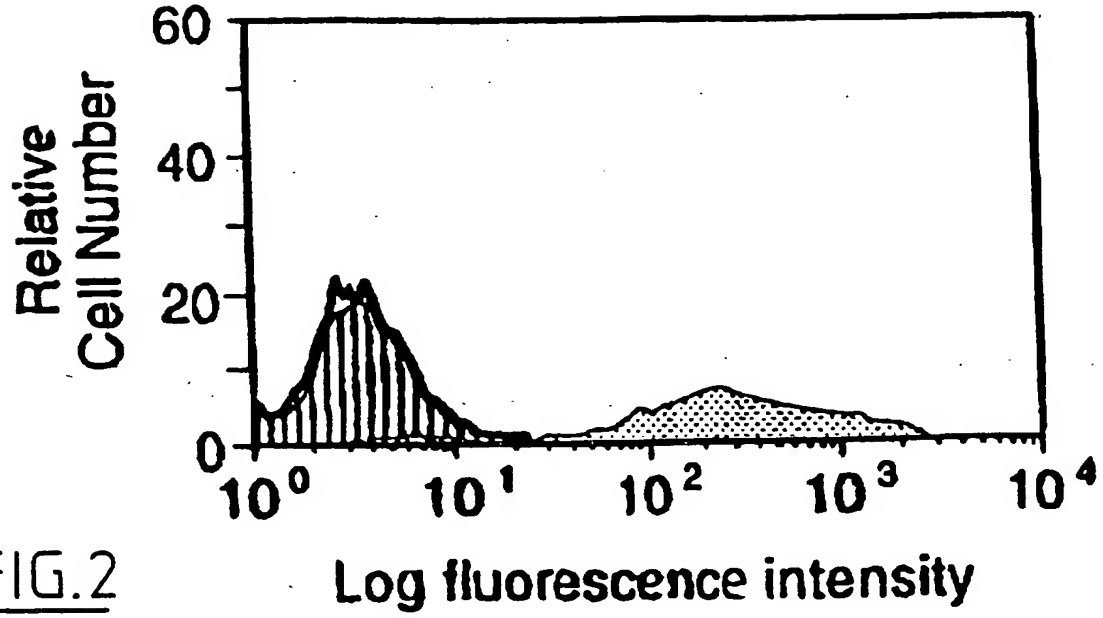
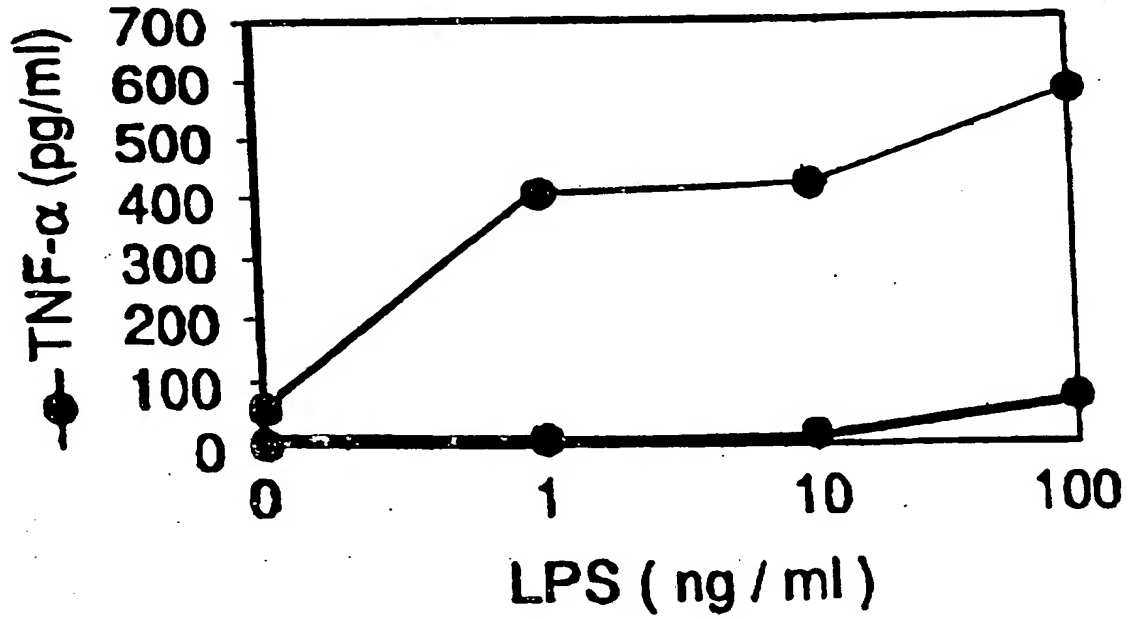


FIG.2



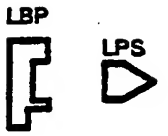


FIG.3A

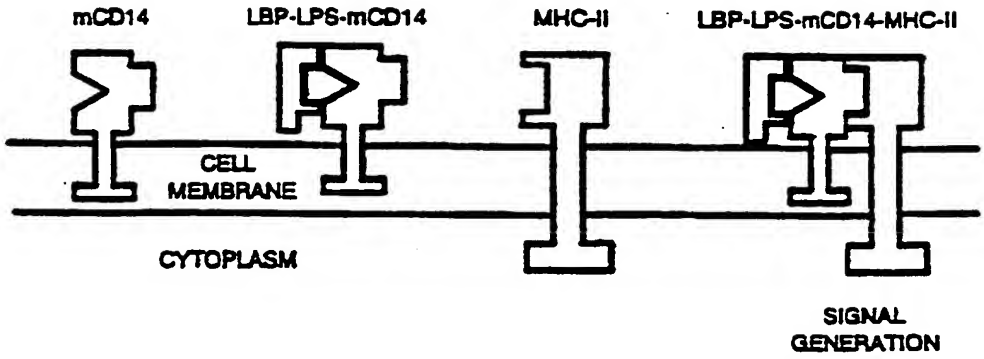
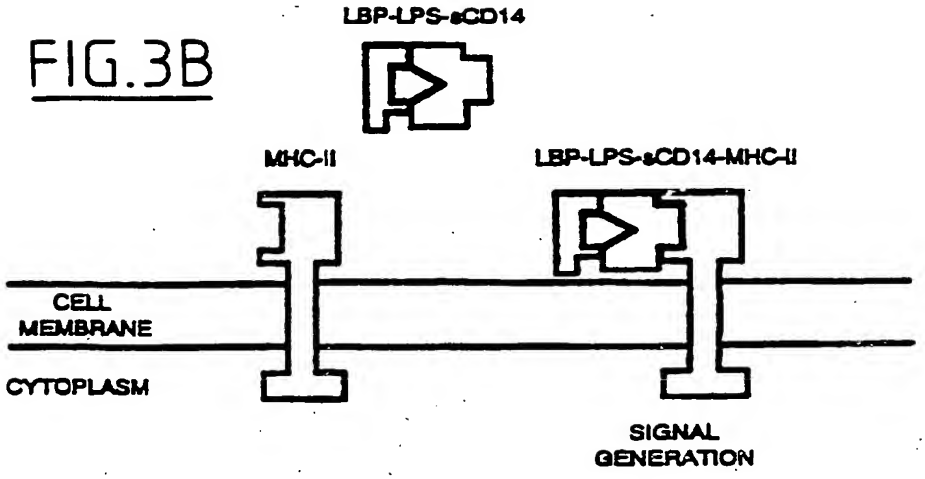
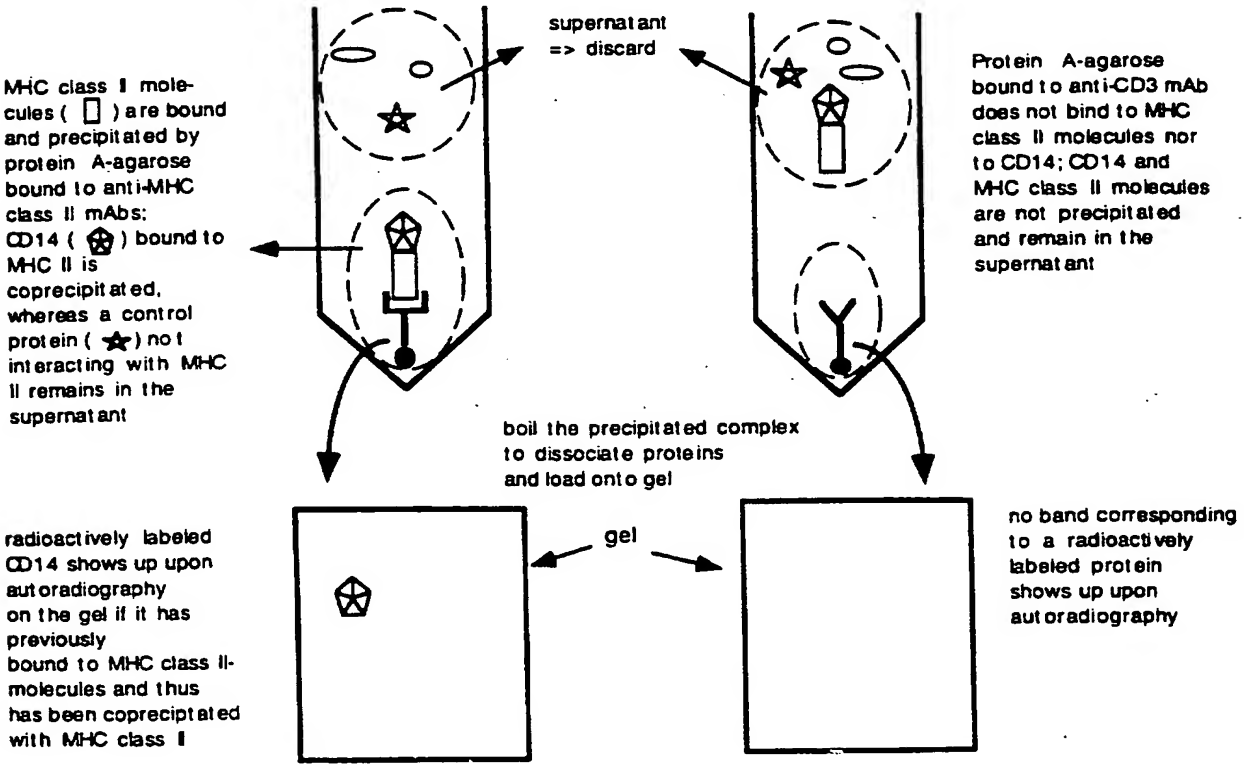


FIG.3B



Coprecipitation of MHC class II-molecules and rCD14: experimental approach



▯ MHC class II molecule

○ other proteins, not interacting with MHC II or CD14

⬠ recombinant CD14 produced by in vitro transcription/translation and labeled with radioactive ^{35}S methionin

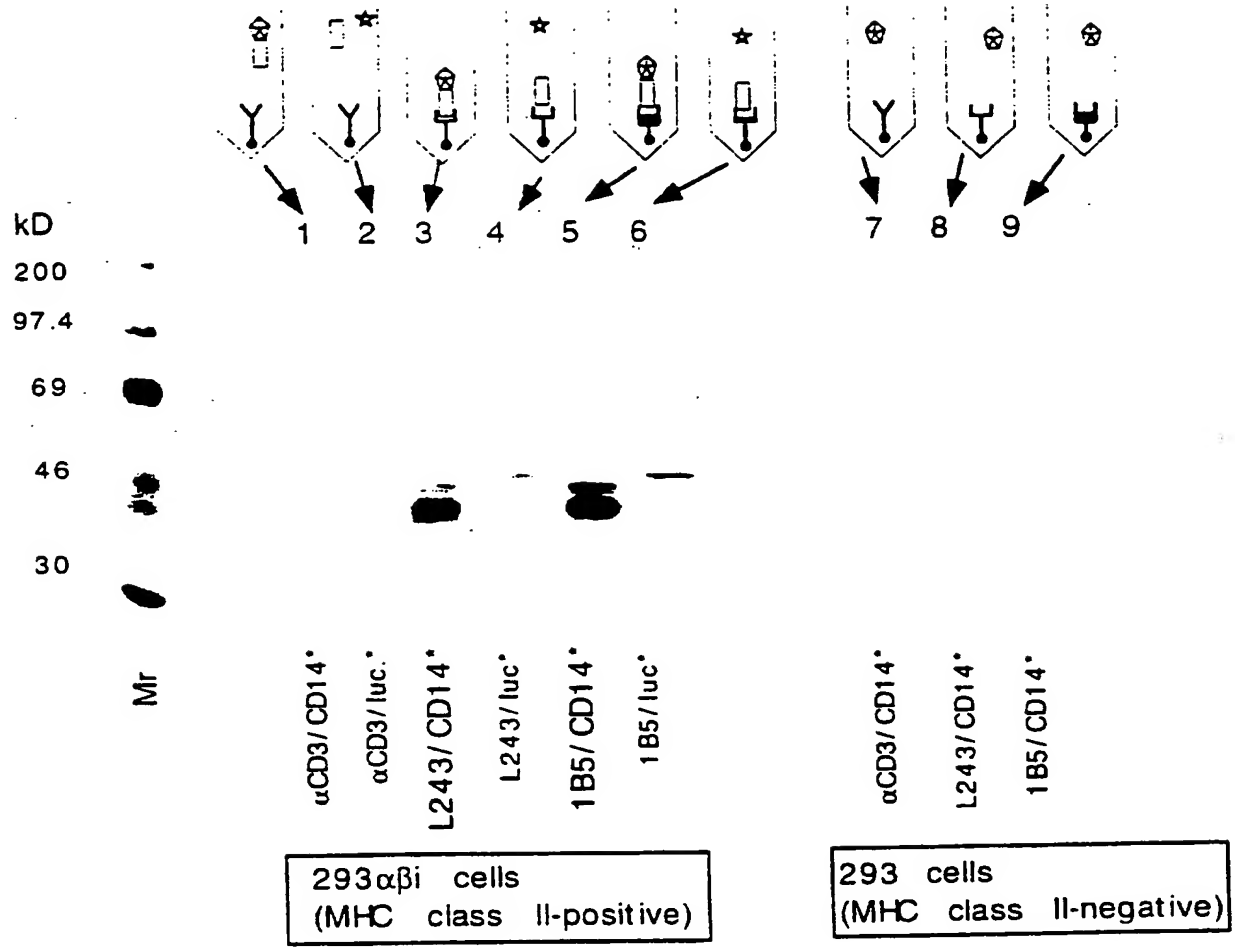
★ recombinant luciferase produced by in vitro transcription/translation and labeled with radioactive ^{35}S methionin (negative control)

anti-MHC class II mAb bound to protein A-agarose

anti-CD3 mAb (negative control) bound to protein A-agarose

FIG.4

FIG.5



MHC class II molecule

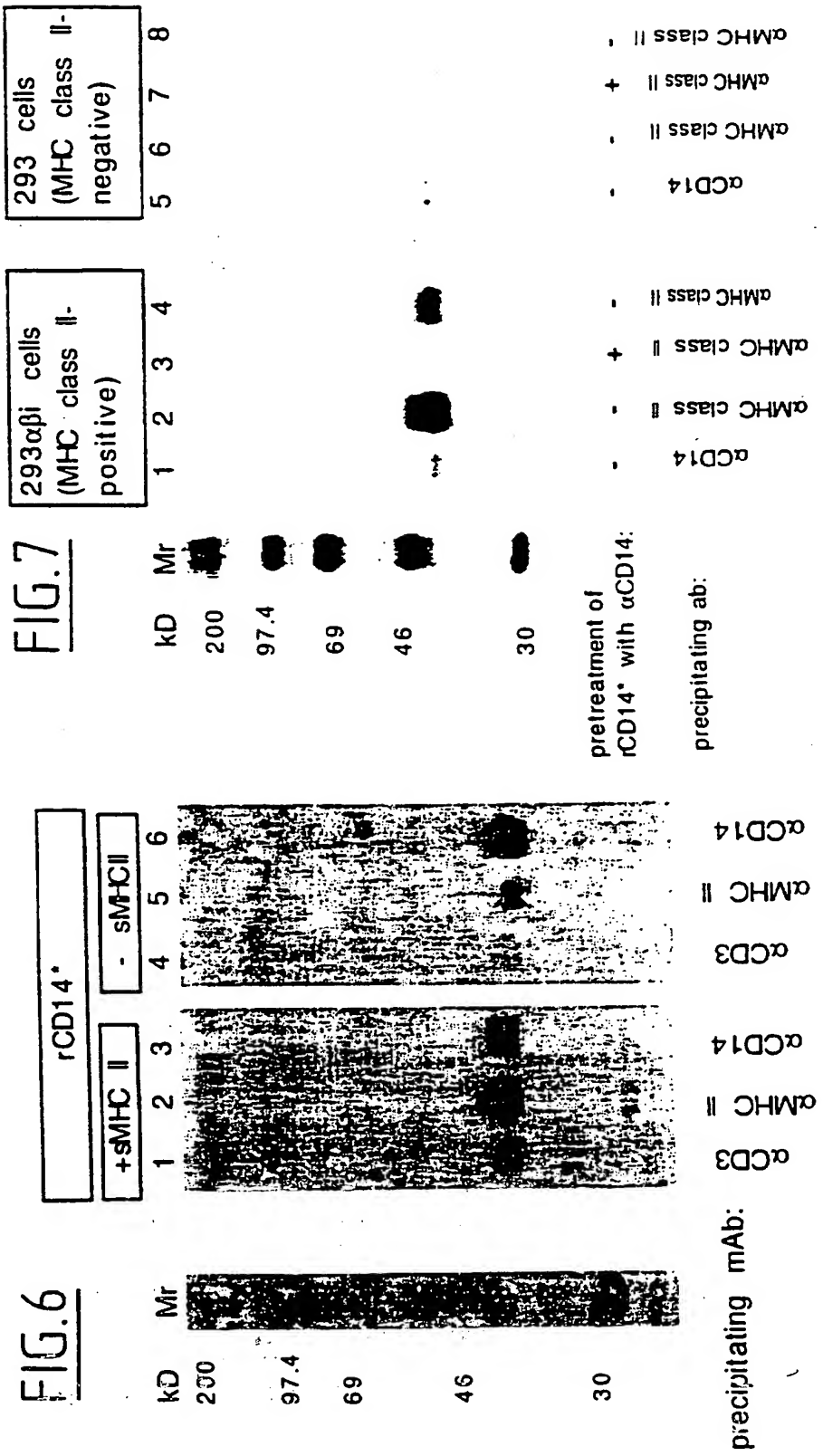
recombinant CD14 (**CD14***)produced by in vitro transcription/translation and labeled with radioactive ^{35}S methionin

recombinant luciferase (**luc.***) produced by in vitro transcription/translation and labeled with radioactive ^{35}S methionin (negative control)

anti-MHC class II mAb (**L243**)

anti-MHC class II mAb (**1B5**)

anti-CD3 mAb (negative control)



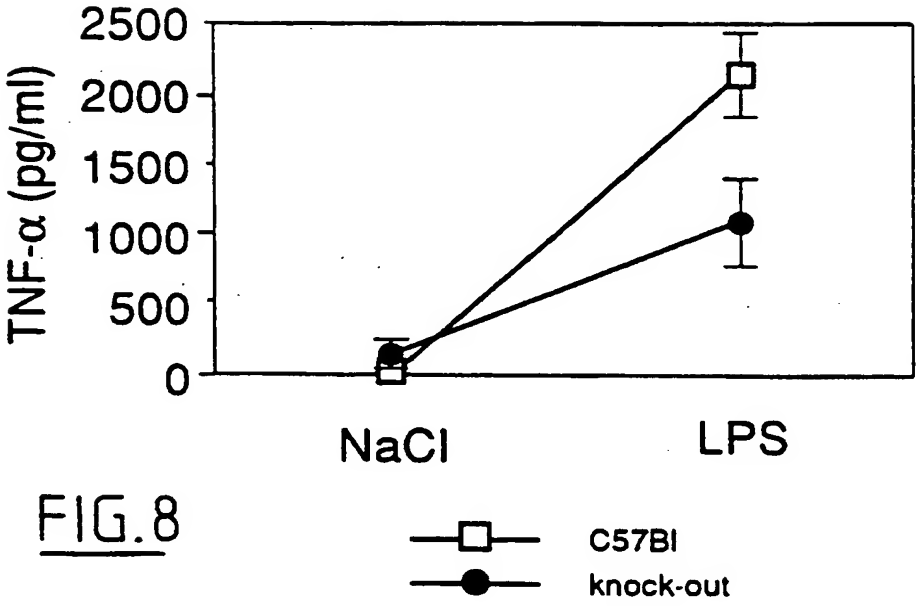

2500
2000
1500
1000
500
0
TNF-α (pg/ml)
NaCl
LPS
C57Bl
knock-out

FIG.8

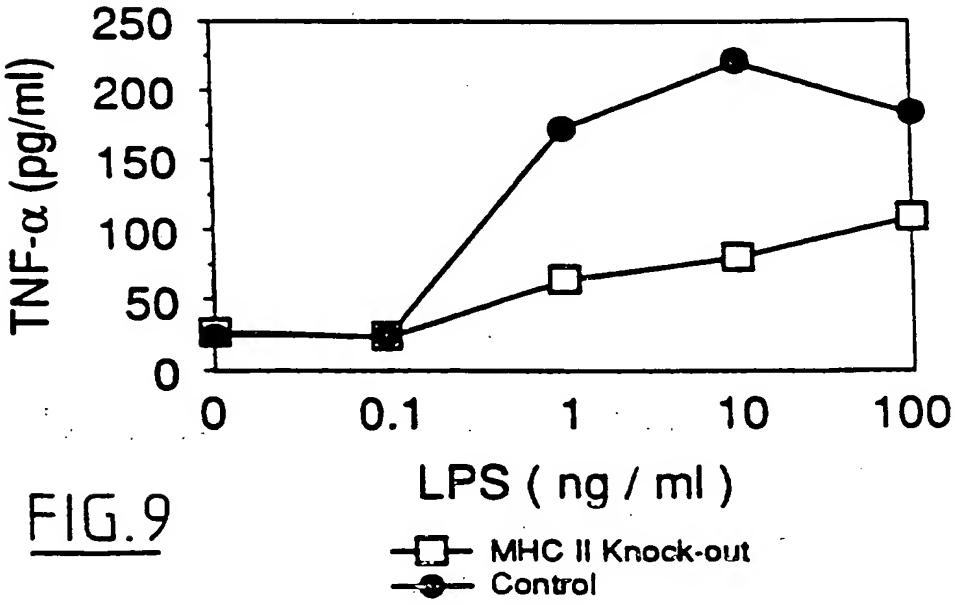

250
200
150
100
50
0
TNF-α (pg/ml)
0
0.1
1
10
100
LPS (ng / ml)
MHC II Knock-out
Control

FIG.9

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(57) Abstract

The invention relates to MHC-II binding and/or MHC-II mimicking molecules for use in interfering in the interaction between an activation stimulus for MHC-II bearing cells, such as phagocytes and cell-bound MHC-II molecules, in the interaction between lipopolysaccharide (LPS) or LPS in a complex with other molecules, such as CD14 and LBP, and cell-bound MHC-II molecules, or in the interaction between products from Gram-positive bacteria or complexes of products from Gram-positive bacteria with molecules such as CD14, and cell-bound MHC-II molecules. The MHC-II binding molecule may be any anti-MHC-II antibody or fragment thereof, or any molecule derived from such an antibody such as humanized, bispecific or other engineered molecules and the like. The MHC-II binding molecule may be selected from the group consisting of CD14, fragments thereof, modified versions thereof, or peptides having MHC-II binding properties.

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INTERNATIONAL SEARCH REPORT

Inte [redacted] pplication No
PCT/E[redacted] 95/05164

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	J. IMMUNOLOGY, vol. 142, no. 9, 1 May 1989, pages 3151-3157, XP000567839 FISCHER ET AL.: "Binding of Staphylococcal enterotoxin A to HLA-DR on B cell lines" see the whole document --- -/--	1,4,5, 10,12, 13,16

[X] Further documents are listed in the continuation of box C.

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X	J. IMMUNOLOGY, vol. 143, no. 8, 15 October 1989, pages 2583-2588, XP000567847 SCHOLL ET AL.: "Staphylococcal enterotoxin B and toxic shock syndrome toxin-1 bind to distinct sites on HLA-DR and HLA-DQ molecules" see the whole document ---	1,2,4,5, 10,13, 14,16
X	J. IMMUNOLOGY, vol. 133, no. 4, October 1984, pages 2104-2110, XP000567848 FORSGREN ET AL.: "The role of I-A/E molecules in B lymphocyte activation : I. inhibition of lipopolysaccharide-induced responses by monoclonal antibodies" see the whole document ---	1-3,5, 10,13-15
X	J. IMMUNOLOGY, vol. 144, no. 3, February 1990, pages 811-815, XP000567849 HAMANO ET AL.: "Direct involvement of surface I-A/E molecules during B-cell maturation using an antigen-specific B cell clone. " see the whole document ---	1-3,5, 10,13-15
X	J. IMMUNOLOGY, vol. 153, no. 8, 15 October 1994, pages 3664-3672, XP000567838 GREY ET AL.: "Selective inhibitory effects of the anticoagulant activated protein C on the responses of human mononuclear phagocates to LPS, IFN-gamma, or phorbol ester" see the whole document ---	1-4,10, 12

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 068 790 (THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY) 5 January 1983 * see whole document, especially page 8 lines 26-34 *	1,2,5,9, 10,12-14
X	WO,A,91 12332 (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) 22 August 1991 * page 3 second to fourth paragraphs * * page 6 paragraph 4 * * page first paragraph * * page 13 last paragraph * see page 14	1,2,5,9, 10,12-14
X	WO,A,94 29451 (CELLTECH LIMITED) 22 December 1994 see page 14 - page 15 * page 37 and 38 paragraph c) * see page 41 - page 42	1,2,4,5, 9,10, 12-14,16
X	EP,A,0 122 814 (MACH B.F.) 24 October 1984 * page 2 last paragraph * * claims 12-14 * * page 12 fourth paragraph * * page 13 last paragraph * * page 14 first paragraph *	1,2,5,9, 10,12-14
X	EP,A,0 204 522 (GEWNETICS SYSTEMS CORPORATION) 10 December 1986 see the whole document	1,2,4,5, 10,13, 14,16
X	DATABASE WPI Section Ch, Week 8851 Derwent Publications Ltd., London, GB; Class B04, AN 365101 XP002001132 & JP,A,63 275 526 (OKAZIMA H.) , 3 May 1987 see abstract	1,2,5,9, 10,13,14
X	J. IMMUNOLOGY, vol. 148, no. 12, 15 June 1992, pages 3943-3949, XP000567850 STEIN ET AL.: "Anti-class II antibodies potentiate IgG2a production by lipopolysaccharide-stimulated B lymphocytes treated with prostaglandin E2 and IFN-gamma" see the whole document	1,3,5,9, 10,12

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/05164

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA,A,2 125 871 (TORAY INDUSTRIES) 28 April 1994 see the whole document	1-4,7-9, 11-14,16
X	WO,A,93 10220 (ANERGEN INC.) 27 May 1993 see page 1 - page 3 see page 20 - page 25	1-5,7-9, 11,12
A		13,14
X	BIOCHEM. BIOPHYS. RES. COMMUN., vol. 193, no. 3, 30 June 1993, pages 1191-1197, XP000578187 PONTZER ET AL.: "Agonist properties of a microbial superantigen peptide" see the whole document	1-4,6,9, 10, 12-14,16
X	PROC. NATL ACAD. SCI., vol. 88, no. 1, 1 January 1991, pages 125-128, XP000578190 PONTZER ET AL.: "Structural basis for differential binding of Staphylococcal enterotoxin A and toxic shock syndrome toxin-1 to class II major histocompatibility molecules" see the whole document	1-4,6,9, 10, 12-14,16
X	MED. MICROBIOL. IMMUNOL., vol. 183, no. 5, August 1994, pages 257-264, XP000578895 HARTWIG ET AL.: "Major histocompatibility complex class II binding site for Streptococcal pyrogenic (erythrogenic) toxin A" see the whole document	1-4,9, 10,13, 14,16
X	BIOCHEM. BIOPHYS. RES. COMMUN., vol. 200, no. 2, 29 April 1994, pages 1059-1065, XP000578181 TORRES ET AL.: "Identification of an HIV-1 NEF peptide that binds to HLA class II antigens" see the whole document	1-4,6,9, 10,13, 14,16

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INTERNATIONAL SEARCH REPORT

Intr[redacted]plication No
PCT/E[redacted]95/05164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 119, no. 1, 5 July 1993 Columbus, Ohio, US; abstract no. 6755w, SOOS ET AL.: "Identification of binding domains of the superantigen, toxic shock syndrome toxin-1, for class II MHC molecules" page 706; column 6757; XP002010928 see abstract & BIOCHEM. BIOPHYS. RES. COMMUN., vol. 191, no. 3, 1993, pages 1211-1217, SOOS:	1-4,6,9, 10,13, 14,16

X	WO,A,94 28025 (THE SCRIPPS RESEARCH INSTITUTE) 8 December 1994 see the whole document	1-4,6-12
A		13-16

X	WO,A,92 04908 (IMTOX PRIVATINSTITUT FÜR IMMUNBIOLOGISCHE FORSCHUNG GMBH) 2 April 1992 see the whole document	1-4,6,7, 9-12
A		13-16

X	WO,A,93 19772 (NORTH SHORE UNIVERSITY HOSPTITAL RESEARCH CORPORATION) 14 October 1993 see the whole document	1-4,6-12
A		13-16

A	IMMUNOBIOLOGY, vol. 182, no. 5, August 1991, pages 449-464, XP000578897 RUPPERT ET AL.: "IL-4 decreases the expression of the monocyte differentiation marker CD14, paralleled by an increasing accessory potency" see the whole document	1-4,9, 10,13-16

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/05164

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. claims 1-4,9,10,12-16 (all partially); 5
2. claims 1-4,9,10,12,16 (all partially); 6
3. claims 1-4, 7-9,11-16 (all partially)
4. claims 1-4, 7-9,11-16 (all partially)

- See continuation-sheet -

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1) claims 1-4,9,10,12-16 (all partially); 5

Anti-MHC-II antibodies or fragments thereof (directed to the LPS or CD14/LPS binding site of a cell), pharmaceutical compositions containing them and their use for interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound MHC-II molecules, and in the treatment of diseases.

2) claims 1-4,9,10,12-16 (all partially); 6

Other MHC-II binding molecules (CD14 and derivatives, fragments, etc. thereof) capable of blocking LPS or CD14/LPS complex binding to MHC-II, pharmaceutical compositions containing them and their use for interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound molecules and in the treatment of diseases.

3) claims 1-4,7-9,11-16 (all partially)

MHC-II mimicking molecules having at least a portion homologues to MHC-II or its soluble form, capable of having MHC-II antagonistic properties and of binding LPS or complexes thereof, pharmaceutical compositions containing them, their use for interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound MHC-II molecules and in the treatment of diseases.

4) claims 1-4,7-9,11-16 (all partially)

MHC-II mimicking molecules not included in the above-mentioned subjects, capable of blocking the MHC-II binding site on LPS or CD14/LPS complexes (including antibodies against LPS or LPS/CD14 complexes, parts thereof), pharmaceutical compositions containing them, their use for interfering in the interaction between an activation stimulus for MHC-II bearing cells and cell-bound MHC-II molecules and in the treatment of diseases.

INTERNATIONAL SEARCH REPORT

information on patent family members

onal Application No
PCT/EP 95/05164

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